



Insect Transmission of Plant Pathogens: a Systems Biology Perspective

 Michelle Heck^{a,b,c}

^aUSDA Agricultural Research Service, Emerging Pests and Pathogens Research Unit, Robert W. Holley Center for Agriculture and Health, Ithaca, New York, USA

^bBoyce Thompson Institute, Ithaca, New York, USA

^cPlant Pathology and Plant Microbe Biology Section, School of Integrative Plant Science, Cornell University, Ithaca, New York, USA

ABSTRACT Insect-vectored pathogens pose one of the greatest threats to plant and animal, including human, health on a global scale. Few effective control strategies have been developed to thwart the transmission of any insect-transmitted pathogen. Most have negative impacts on the environment and human health and are unsustainable. Plant pathogen transmission by insect vectors involves a combination of coevolving biological players: plant hosts, insect vectors, plant pathogens, and bacterial endosymbionts harbored by the insect. Our ability to help growers to control vector-borne disease depends on our ability to generate pathogen- and/or disease-resistant crops by traditional or synthetic approaches and to block pathogen transmission by the insect vector. Systems biology studies have led to the reexamination of existing paradigms on how pathogens interact with insect vectors, including the bacterial symbionts, and have identified vector-pathogen interactions at the molecular and cellular levels for the development of novel transmission interdiction strategies.

KEYWORDS aphids, citrus greening, plant virus, proteomics, psyllids, transcriptomics, vector biology

Insect vector biology refers to the field of study focused on the evolutionary, anatomical, physiological, cellular, and molecular mechanisms involved in the transmission of pathogens by insect vectors. Most insect vectors of plant pathogens are hemipteran insects, e.g., aphids, whiteflies, and psyllids, that have piercing sucking mouthparts in common. All have a coevolved, mutualistic relationship with intracellular, often obligate, microbial partners known as endosymbionts. Although gene silencing in hemipteran insects has been reported, many are not easily amenable to transgenic manipulation. Genome sequencing of multiple hemipteran insects and their endosymbionts has enabled a deeper understanding of the metabolic interplay between the insects and their bacterial endosymbionts and how these insects colonize their plant hosts (1–5), but the mechanisms regulating pathogen transmission remain elusive from the analysis of the genome sequencing data alone. Genome sequencing did, however, set the stage for proteomics and phenotyping research to understand the complex, dynamic, tritrophic interactions among insects, plant pathogens, and plant hosts, including the regulation of pathogen transmission. Research on molecular interactions between insect vectors and plant pathogens has the promise to lead to the discovery of novel disease management strategies that block pathogen transmission by insects.

Many plant pathogens are transmitted by hemipteran insects in a circulative manner, meaning that the pathogen is ingested and moves throughout the body of the

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Address correspondence to mlc68@cornell.edu.

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 Bugs in bugs: @HeckLab_BTI is using systems biology to study how insects transmit plant pathogens

insect prior to transmission to a new host plant (6). Although the precise route of pathogen movement through the vector tissues may vary among different pathogens, circulative plant pathogens share many biological, biochemical, and ecological features (6). Circulative plant pathogens induce physiological changes in their plant hosts resulting in behavioral changes in the insects that optimize their plant-to-plant spread (6–8). Plant pathogen transmission is regulated at the molecular level by a suite of spatially and temporally controlled protein interactions. Knowledge of these complex interactions allows for the development of tools to block transmission, to rapidly identify important vector populations, and to improve disease management. Blocking pathogen transmission is the next frontier of precision vector-borne disease management strategies (9, 10).

Aphids have become the most tractable vector for molecular studies on virus transmission because of their ability to switch between sexual and parthenogenetic reproduction and their fascinating variation in the ability to transmit plant viruses (11). Sexual morphs can be mated for genetic analysis of phenotypes of interest, for example, virus transmission, and phenotypically interesting lines, for example, efficient vectors that transmit a particular virus very well and nonvectors that do not transmit the same virus, can be reared asexually in the parthenogenetic mode as clones indefinitely (12). Identification of aphid proteins that regulate virus transmission has been a major research focus for the past decade (6). Proteomics was used to identify proteins that can differentiate among aphid populations and individuals that vary in their efficiency of virus transmission (11, 13). Proteomics was also used to reveal genetic heterogeneity in the aphid's obligate intracellular bacterial symbiont, the proteobacterium *Buchnera aphidicola*, in clonal lineages of aphids (11), a surprising discovery given that *Buchnera* is vertically transmitted from mother to offspring. One *Buchnera* genotype was always found in higher abundance in efficient vector aphid genotypes, indicating a synergistic relationship between the genotype of aphid and the genotype of *Buchnera* in the determination of virus vectoring ability (11). *Buchnera* has an extremely reduced genome and cannot be cultured (5). These features have limited the use of *Buchnera* as a means to develop field-deployable transmission-blocking strategies. New approaches to manipulate *Buchnera* genes and gene expression directly inside the aphid may be a future avenue of research to consider.

The host plant of the aphid influences the virus transmission efficiency of aphids. Serendipitously, an effect of the host plant on virus transmission efficiency was discovered when a clone of the aphid *Myzus persicae*, which had been reared on *Physalis floridana* and used as the principal vector in *Potato leafroll virus* (PLRV) transmission studies for many years, was reared on turnip plants (14). Unexpectedly, this host switch impaired the ability of turnip-reared aphids to transmit PLRV. The aphid gut is the first site of entry for circulative viruses into aphid tissues. A combination of whole-insect proteome analysis, confocal microscopy with fluorescently labeled antibodies of aphid gut tissue, and enzyme activity assays was used to show that turnip plants induce an increase in the expression and activity of the cysteine protease cathepsin B in *M. persicae* compared to when the aphids are reared on *physalis*. Oral delivery via artificial diets of the cysteine protease inhibitor E-64 into *M. persicae* reared on turnip plants restored the ability of the aphid to efficiently transmit PLRV in an E-64 dose-dependent manner (14). This work shows that host plants regulate virus transmission by insects and describes the underlying organismal, enzymatic, and cell biological mechanisms.

Vectoring efficiency is developmentally regulated in many insect vectors, and such is the case for *Diaphorina citri* (the Asian citrus psyllid), the insect vector of the Gram-negative bacterium "*Candidatus Liberibacter asiaticus*." "*Ca. Liberibacter asiaticus*" is a bacterium associated with huanglongbing (HLB), also known as citrus greening disease. HLB is the most serious, deadly disease of citrus and is entrenched in all of the citrus-growing counties in Florida. Isolated pockets of infection have now been reported in Texas and California as well. "*Ca. Liberibacter asiaticus*" is currently unculturable. In two studies conducted in Japan and Florida (15, 16), only *D. citri* insects that acquired "*Ca. Liberibacter asiaticus*" as nymphs, but not adults, were able to transmit

"*Ca. Liberibacter asiaticus*" to healthy citrus trees. Nymphs show an attenuated proteome response to being reared on trees infected with "*Ca. Liberibacter asiaticus*" compared to that of adults (17). Microscopic analysis shows that "*Ca. Liberibacter asiaticus*" induces nuclear DNA fragmentation in adult midgut epithelial cells associated with apoptosis (18, 32). A dual quantitative transcriptomic and proteomic analysis of excised *D. citri* guts showed that "*Ca. Liberibacter asiaticus*" induces changes consistent with an apoptosis proteomic phenotype, including discordance in transcript and protein abundance of mitochondrial and protein translation enzymes (19) resulting in a concerted downregulation of the tricarboxylic acid cycle enzymes. *D. citri* nymphs are resistant to the detrimental cellular effects that are induced by "*Ca. Liberibacter asiaticus*" in adults (32). These findings parallel the invasion of the midgut of the mosquito (genus *Anopheles*) vector by *Plasmodium* species. Invasion of the mosquito midgut by the *Plasmodium* parasite causes cellular damage that activates a cascade of responses leading to apoptosis. More than 80% of the invading parasites are destroyed by the mosquito immune response during the process. Reactive oxygen species are released that are toxic to the *Plasmodium* cells (reviewed in reference 20). We hypothesize that a similar response occurs in adult *D. citri* in response to "*Ca. Liberibacter asiaticus*," and the nymphs do not mount this immune response, perhaps to allow establishment of the *D. citri* bacterial symbionts. Acquisition and transmission differences between adults and nymphs have significant implications regarding disease epidemiology and control of this economically important citrus pathogen. New biological tools are needed to study these differences and exploit them for novel transmission control strategies.

A complete understanding of vector-pathogen interactions during transmission demands an understanding of pathogen interactions within the host plant. Evidence suggests that insect-borne plant pathogens use similar protein interactions for intra- and intercellular movement in plants and insects and have been selected to alter their host plants in ways that maximize transmission by insects. In many ways, plants are easier to use for protein interaction studies than insects, so we frequently optimize new methods in plants that we later apply to the study of interactions in insects. Our lab has been using a transformative chemical cross-linking high-resolution mass spectrometry strategy called protein interaction reporter (PIR) technology to make measurements of virus-virus and host-virus protein-protein interactions. The protein interactions are measured *in vivo* while protein complexes are intact. Then, because of the advanced molecular design of the cross-linking molecule, measurements of the protein interactions on a proteome-wide scale are made by using the technology and informatics workflows optimized for peptide identification by tandem mass spectrometry. Our first PIR measurements of infectious PLRV virions were modest (21), including protein interaction topologies within and between the two viral structural proteins, but physical measurements of the virion were a first for this family of viruses. Coupled to the phenotypic analysis of virus mutants, these data revealed the disordered protein interaction topologies within the virion responsible for capsid stability, infection of plants, and movement within aphids. We expanded on this initial study to generate the first virus-host interactome for this virus in plants where the topologies of every protein interaction were mapped (22). Quantitative affinity purification mass spectrometry experiments (23, 24) with PLRV and PLRV mutants coupled to the PIR data and functional analysis of plant host proteins in the network revealed a complex regulatory network for the structural proteins regulating virus titer and systemic movement in plant hosts. These studies revealed, among other findings, the plant chloroplast as a key, yet previously unexplored, plant-virus interface that is actively manipulated by the virus during infection. Interestingly, although not surprisingly, our unpublished data show that HLB-infected citrus trees also exhibit changes in chloroplast metabolism. Given the close relationship between photosynthesis and breeding efforts to maximize yields in agricultural crops, understanding how pathogen-induced changes in chloroplast function influence pathogen transmission by insects is an important aim of future research.

Our ability to help growers to control vector-borne diseases depends on our ability to generate pathogen- and/or disease-resistant crops by traditional or synthetic approaches and to block pathogen transmission by insect vectors. Achievement of these goals is hampered in part because (i) the vast majority of circulative plant pathogens, such as “*Ca. Liberibacter asiaticus*,” are not culturable and (ii) the insect vectors are not easily amenable to genetic manipulation. One major development in this area would be the use of synthetic biology tools to create culturable forms of these recalcitrant, fastidious plant pathogens that will allow for genetic and experimental manipulation and foreign gene expression in plants and insects. Such an achievement is not out of reach, as the first synthetic bacterium was brought to life by using a chemically synthesized genome (25). Our lab is part of a team of researchers now working to achieve this goal for “*Ca. Liberibacter asiaticus*.” Another major development will be in our ability to genetically modify the insect vector to shut down the genes that regulate pathogen acquisition, replication, and/or transmission. The use of synthetic, naturally occurring, or hybrid endosymbionts could facilitate this goal directly (26–28), or plant virus vectors may be used to indirectly modify gene expression in the insect (29), as can new applications of gene editing technologies. The use of bacterial endosymbionts to directly modify the expression of or disarm genes in an insect vector host to block transmission is a distinct strategy from efforts using *Wolbachia* to modify virus transmission phenotypes in mosquitoes. There are still limitations to the use and potential long-term efficacy of the latter strategy (30). Ideally, a vector control strategy that did not kill the insect but rather blocked transmission would not be subject to the development of resistance like traditional insecticides and could stop the spread of vector-borne pathogens. However, such a strategy may not be totally straightforward because circulative pathogens often impart fitness benefits to their insect vectors, including *D. citri* and “*Ca. Liberibacter asiaticus*” (31). Systems biology and genetic studies by my lab and others have already pinpointed which molecular pathways and bacterial symbionts involved in vector-pathogen interactions would be ideal targets for such approaches.

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